10/525589
DT15 Rec'd PCT/PTO 25 FEB 2005

BIOSENSOR

Field of the Invention

The present invention relates to a system for detecting a physical, chemical or biochemical reactions, and in particular to a system in which surface electromagnetic waves (SEWs) interact with a specimen involved in the reaction.

Background to the Invention

Biosensors incorporating surface electromagnetic wave technology (and, in particular, surface plasmon resonance - SPR - sensors) are increasingly gaining popularity in pharmaceutical, medical and environmental applications as well as in biochemical research. These type of sensors require no labelling and offer the possibility of real-time monitoring of binding events. They are based on the sensitivity of surface electromagnetic waves (SEW) to the refractive index of the thin layer adjacent to the surface where the SEW propagates. In a typical biosensor application, one binding partner is immobilized on the surface (often called a target) and the other partner is flowed across it. As binding occurs, the accumulation or redistribution of mass on the surface changes the local refractive index that can be monitored in real time by the sensor.

Several methods of SPR registration have been proposed and realized in biosensors. The most popular methods are based on Kretschmann-Raether configuration where intensity of the light reflected from sensor is monitored. This technique, considered to be one of the most sensitive, is described in J. Homola et al, Sensors and Actuators B 54, p.3-15 (1999) and has a detection limit of $5x10^{-7}$ refractive index units. Measuring SPR phase changes can further increase the sensitivity of the sensor by one or two orders of magnitude. This is described in Nelson et al, Sensors and Actuators B 35-36, p.187 (1996) and in Kabashkin et al, Optics Communications 150, p.5 (1998). Prior art interferometric devices such as a Mach Zehnder device have been configured to measure variations in the refractive index at the sensor surface via phase shifts. This is disclosed in WO01/20295. The configuration requires four independent components and is sensitive to sub-wavelength relative displacements of these components and hence very small mechanical and environmental perturbations. A mechanically more robust monolithic interferometric design is outlined in WO03014715.

However, although the theoretical limit for the sensitivity can be as good as 10^{-8} refractive index units, the sensitivity of real systems is limited to 10^{-6} due to fluctuations in the temperature and chemical composition of the buffer surrounding the sample. For example, to achieve a sensitivity of 10^{-7} refractive index units a temperature stability better than 10^{-3} °C would be required. This is due to the fact that the influence of changes in the refractive index of the surrounding buffer cannot be isolated from the influence of changes in thickness and refractive index of the analyte absorbed on sensor surface using the methods and systems of the prior art.

Summary of the Invention

An object of the present invention is to provide a surface electromagnetic wave (SEW) sensor system that can compensate for changes in the bulk refractive index of a buffer or allows the contribution of the bulk refractive index to an interference pattern to be separated from the contribution of an analyte absorbed on the sensor surface. The invention relates to a sensor system, a sensor method and carrier chip designs for use in a sensor.

According to a first aspect of the present invention a system for detecting a physical, chemical or biochemical reaction comprises:

a coherent radiation source for producing an incident wave;

a carrier surface for supporting a specimen to be analysed, the carrier surface mounted on a substrate and capable of supporting surface electromagnetic waves (SEW);

means for splitting the incident wave into an SEW and a first scattered wave, wherein the SEW propagates along the carrier surface and interacts with the specimen;

means for generating a second scattered wave from the SEW; and,

a detector for monitoring the interference between the first scattered wave and the second scattered wave.

According to a second aspect of the present invention a carrier chip for a specimen to be monitored, comprises:

a dielectric substrate; and

a conductive film formed on the surface of the substrate suitable for carrying the specimen;

wherein the conductive film comprises first means for splitting an incident wave into a first scattered wave and a surface electromagnetic wave (SEW), the

SEW propagating along the carrier surface and interacting with the specimen, and a second means for generating a second scattered wave from the SEW.

According to a third aspect of the present invention a method of monitoring a specimen undergoing a physical, chemical or biochemical reaction occurring on a surface supporting surface electromagnetic waves (SEW), comprises the steps of:

splitting an incident wave into a first scattered wave and SEW such that the SEW propagates along the surface and interacts with the specimen;

splitting the SEW which has interacted with the specimen to generate a second scattered wave; and,

monitoring the interference pattern between the first and second scattered waves.

Brief Description of the Drawings

Examples of the present invention will now be described with reference to the accompanying drawings in which:

Figure 1 is a schematic illustration of an apparatus according to the present invention for detecting a physical, chemical or biochemical reaction;

Figure 2 illustrates a first embodiment of a system according to the present invention in which changes in bulk refractive index are compensated for a particular angle so that the system is only sensitive to the changes in thickness or refractive index of an analyte absorbed on the sensor surface;

Figure 3 illustrates a second embodiment of a system according to the present invention;

Figure 4 shows detail of a carrier chip according to the present invention;

Figure 5 shows another embodiment of a carrier chip according to the present invention;

Figure 6 shows the calculated variation of the interference fringe position at the optimal angle in the embodiment of Figure 2 versus bulk refractive index of a surrounding buffer;

Figure 7 illustrates measurements made using the embodiment of Figure2 where the buffer (water) surrounding the chip was cooled down from 46 °C to 22 °C. The peak position ("phase") is insensitive to the bulk refractive index changes associated with heating while peaks separation ("frequency") is.

Figure 8 illustrates a further carrier chip according to the present invention;

Figure 9 illustrates an embodiment of a multi-point array detection system system according to the present invention in which the whole sensor surface is simultaneously illuminated using a line source and a 2-D CCD-array is used for detection;

Figure 10 shows an example of an image observed on the CCD-array shown in Figure 9;

Figure 11 shows multi-track analysis of streptavidin binding to a carboxymethylated surface along the sensor line. Tracks on the graph correspond to points along the sensor line separated by approximately 40 μm; and,

Figure 12 shows an embodiment of a 2-D sensor array.

Detailed Description

Figure 1 shows schematically a system for monitoring a physical, chemical or biochemical interaction in accordance with a first aspect of the present invention. A coherent optical beam generated by a monochromatic laser is focused using a lens, onto the edge of a metallic film able to support surface electromagnetic waves (SEWs). The optical beam passes through the glass prism on which the metallic film is mounted. A near-infrared laser 11 is used as the illumination source. Using a near-infrared source has the advantage of long propagation length for surface plasmons in gold and silver while conventional optics can be still used for imaging and illumination. However, other monochromatic sources are suitable and may be used.

The laser provides a p-polarised beam. The p-polarised laser beam passes through the focusing lens 12 and then through the glass prism 13 on which a substrate 14 with a microfabricated metal film is attached, using an index matching liquid or gel in a fluidic cell. The index matching gel reduces light scattering and creates a continuous optical path. The glass prism may be a triangular prism as shown or a hemi-cylindrical prism. The laser beam is focused on an edge of the structure 13. The laser beam falls on the glass/liquid interface at an incidence angle larger than the angle of total internal reflection, so that the laser beam is totally reflected except at a small area around the edge of the metal structure. At the edge of the structure the evanescent light wave formed on reflection is partly scattered into light 15 propagating through the fluidic cell and partly scattered into a plasmon wave 16 propagating along the metal structure. The plasmon wave is further scattered by the structure to produce light wave 17. Waves 15 and 17 propagate

through the liquid cell and produce an interference fringe pattern on the measurement device 18.

The metal structure can be formed from gold or silver, or any other metal capable of supporting surface plasmons or a combination of them, or alternatively a dielectric multilayer supporting a SEW. It is preferred to use either gold or silver/gold multilayer to increase surface plasmon propagation length. The metal structure can be deposited on the prism using a lithographic process. The metal structure is described below in more detail with reference to Figures 4 and 5 below.

The method of the measurement is further illustrated by Figure 2. As the phase is conserved during scattering processes the volume radiation beams 25 and 27 can be brought to interference. The phase difference between the beams depends on a surface plasmon (or SEW) wave vector k_{sp}, the distance between the two scattering points *a* and the refractive index of the solution *n*. As the refractive index of the solution *n* and plasmon wave vector k_{sp} change, the shift of the interferogram will be detected by a sensor 28 that can be either 2-section photodiode, 1-D photodetector or CCD array, or 2-D photodetector or CCD array.

The phase difference between beams 25 and 27 can be written as

$$2\cdot\pi\cdot a\cdot(n_{sp}-n\cdot\cos(\theta))/\lambda\ ,$$

where λ – is the wavelength of the light and n_{sp} is related to *n* via following equation:

$$n_{sp}=\sqrt{\frac{\varepsilon_m\cdot n_l^2}{\varepsilon_m+n_l^2}}\ .$$

The bulk refractive index *n* of the buffer fluid and the local refractive index n_l next to the metal surface are distinct and can differ due to layers physically or chemically absorbed on the metal surface (i.e. bound analyte). It can be assumed that:

$$n_l=n+\Delta n\ .$$

The direction to any particular point on the interference pattern is:

$$\cos(\theta)=\frac{1}{n}(n_{sp}-(m+\Delta m)\cdot\lambda/a)\ .$$

where *m* is an integer for a maximum, a half-integer for a minimum, or any other number for an arbitrary fixed point on the interference pattern and Δm is an additional phase shift upon excitation and detachment of a SEW. The direction θ of this particular point depends both on *n* and Δn, but expanding the above equation

into a series and differentiating it can be found that changes in bulk refractive index in this geometry are partly compensated and the system is more sensitive to the refractive index changes on the surface by a factor of:

$$\frac{\partial\theta / \partial n}{\partial\theta / \partial\Delta n} \approx \frac{n^2}{\varepsilon_m}$$ (this factor is of order 10 for gold and silver).

Full immunity to the bulk refractive index variation cannot be achieved as the optical path in the solution $n^*|AD|$ is always smaller than the one along the metal structure $n_{sp}{}^*a$, as shown in Figure 2.

Nevertheless, this suppression factor can be further improved or n and Δn separated by varying the shape of the fluidic cell. For example the optical path length of the interfering rays for a particular point m can be equalized as shown in Figure 2. The direction to the detector θ' is connected to θ via Snellius law:

$$n \cdot \sin(\theta + \varphi) = \sin(\theta' + \varphi)$$

where Φ is the tilt angle of the cell's exit wall. This equation can be used to find an angle θ where the variation in n is not reflected in θ' by solving:

$$\frac{\partial}{\partial n}(n \cdot \sin(\theta + \varphi)) = 0.$$

The solution is:

$$\varphi_m = \arctan\left(\frac{(m+\Delta m)\lambda - a n_{sp}^3 / \varepsilon_m}{\sqrt{a^2 n^2 - (a n_{sp} - (m+\Delta m)\lambda)^2}}\right) - \arccos\left(\frac{a n_{sp} - (m+\Delta m)\lambda}{a n}\right).$$

At such a tilt angle Φ a small variation of n will not change the direction θ' on the given point m. The calculations illustrating this are shown in Figure 6. As can be seen from Figure 6, significant variation of buffer refractive index (equivalent to heating the water surrounding the sample by ~100°C) produces a negligible shift (within 1µm) in the fringe position at the optimal angle. On the other hand the sensitivity to the variation in Δn will stay the same.

We can also find an optimum number m for a given value of Φ. For example for the right wall (Φ =0):

$$m \approx 2\frac{a n^3}{\lambda|\varepsilon_m|} \text{ and } \theta = \arccos\left[\left(\frac{\varepsilon_m + n^2}{\varepsilon_m}\right)^{3/2}\right].$$

For silver film (ε_m =-53) surrounded by water (n=1.326) the optimal angle θ≈18°. The part of the fringe pattern located at the optimal angle will not move if only the bulk refractive index varies and the rest of the pattern will breathe around it. Figure 7

shows the experimentally observed variation of a peak position at the optimal angle while cooling the water surrounding the silver microstructure from 46°C down to 22°C (refractive index variation ~$2*10^{-3}$). The lower line shows that the peak position (phase) is insensitive to bulk refractive index changes, whilst the upper line shows that the peak separation (frequency) varies considerably.

Alternatively, the bulk refractive index can be measured simultaneously and subtracted during data analysis. In the case of a semi-cylindrical cell as illustrated in Figure 3, where the first edge of the structure is aligned to the geometrical center of the cell, the interference fringes on the detector 38 are equidistant (this simplifies harmonic analysis of the pattern) and the distance between interference fringes ("frequency") depends only on the refractive index of the buffer while fringe position depends both on n and Δn.

Figure 4 shows a close up of the profile of a film for use in the apparatus of Figure 1, Figure 2 or Figure 3 in accordance with a second aspect of the present invention. As shown in Figure 4 a carrier film is mounted on the surface of a supporting transparent dielectric material. In this embodiment the carrier film includes a first section 41 of a first thickness and a second section 42 of greater thickness. Coherent radiation 43 incident on the edge of the first section in an attenuated total reflection geometry (ATR), i.e. incident under angle larger than the angle of total internal reflection, will partly scatter into volume radiation 45 and partly generate a SEW 44. This SEW 44 will propagate along section 41 of the carrier film until it reaches the boundary between sections 41 and 42. At this boundary the SEW will again scatter generating a volume radiation 46.
In another embodiment shown in Figure 5, coherent incident radiation 51 is incident on a flat part 52 of a carrier film generating SEW 53 which travels along the section 52 towards the edge of a second section 54. There it is partially scattered into volume radiation 55 and partially transmitted along section 54. The SEW will further travel to the opposite edge of the section 54 where it will be scattered into volume radiation 56.

Further film designs are possible, incorporating the features of both Figures 3 and 4. It is also possible to induce scattering from the surface of the film by introducing a change in the refractive index of the film or surrounding materials at the point at which scattered waves are to be generated. Different means of SEW generation known in the art can be envisaged such as using small apertures or arrays of apertures or using gratings.

It is also envisaged that the SEW could be routed along the carrier surface and/or focused on an area of interest using plasmonic circuitry known in the art. This is described in: F.R. Aussenegg et al., Opto-electronic review 10, p.217 (2002). Plasmonic circuitry to this end could be formed lithographically.

Figure 8 shows another possible embodiment of a microfabricated sensor with a built-in reference area. The incident beam is scattered not only on the metal film 81 (as in Fig. 4) but also on the film 87 (which can be both metallic or non metallic) generating a third volume wave 88. The relative phase of the wave 88 will depend on the bulk refractive index only. If the gap between 81 and 87 is different from the length of 81, the contribution of the wave 88 to the fringe pattern can be separated by harmonic analysis.

The above described system can be readily converted into a multi-point array detection system. A possible embodiment of such system is shown on Figure 9. A laser beam 92 generated by a laser 91 goes through a beam expander and conditioner 93 and is focused into a line by the cylindrical lens 94. Scanning mechanism 95 can switch the laser line between a number of microfabricated structures located on a substrate 96. The interference pattern generated by the microstructures is imaged and projected on a CCD-camera 98 by an optical system 97. In the particular embodiment of the optical system described, the image on the CCD-camera is composed of a set of interference patterns generated by every point along the structure. This is shown in Figure10. As there is one to one correspondence between the interference patterns on CCD and points along the structure, every particular location can be traced during a biochemical experiment. This is shown in Figure 11. The system can be used to monitor binding of different analytes to target areas in DNA or protein arrays. In this case, the substrate can be spotted with different targets, illustrated schematically in Figure 12, where target material 121 is spotted on a plurality of microstructures 122 fabricated, for example, according to embodiments of Figures 4, 5. These microstructures can be interrogated either sequentially or simultaneously.

It is further recognised that if the microstructures have different width, as shown in Figure12, the spatial frequency of the interference patterns they produce will be different and their individual signals can be separated by harmonic analysis.

The above described system is particularly suitable for detecting the generation of the complementary base pairs in a strand of DNA. A complimentary DNA strand can be produced using a polymerase and a parent DNA strand. A DNA

strand is built from four base blocks, and binding of each of these four blocks to a DNA strand will lead to a characteristic charge distribution in a polymerase on the surface of the film. This in turn will lead to a characteristic change in the phase velocity of the SEW and hence a characteristic change in the interference pattern. The use of surface plasmon resonance in the detection of nucleotide incorporation during DNA synthesis, is disclosed in WO-A-99/05315, the content of which is hereby incorporated by reference.

In order to increase accuracy a number of identical specimens can be placed along the length of the film so as to give rise to a greater interaction length between the specimen and the plasmons. Characteristic phase changes for particular reactions can be found by monitoring known reactions under known conditions.